The latest WHO reports on infertility show a significant increase in the number of infertile couples worldwide. It is estimated that infertility currently affects about 15-20% of couples, with the male factor accounting for 20-70% of all cases and being significantly higher in developed countries. Oxidative stress is thought to be a major cause of male infertility. It has been suggested that the percentage of infertile men with an red-ox imbalance in semen is as high as 80%. Uncontrolled increase in reactive oxygen species (ROS) in semen may cause irreversible changes to biologically important sperm cell structures by damaging lipids and proteins in cell membranes, decreasing mitochondrial activity and increasing sperm DNA fragmentation. The extent of the damage depends on the condition of the antioxidant defense system, which is responsible for neutralizing the excess of ROS. The antioxidant defense system relies on the action of both enzymatic components and non-enzymatic low-molecular-weight antioxidants such as ascorbic acid (vitamin C), tocopherol (vitamin E) and retinoids (vitamin A). Although the results of both experimental and clinical tests have shown a significant reduction of low-molecular-weight antioxidants in the seminal plasma of infertile men, the evidence for the efficacy of their supplementation in the treatment of male fertility disorders remains inconclusive. In light of the above, this project aims to verify the hypothesis linking oxidative stress with male infertility and to explain the potential protective effect of vitamin C on spermatozoa.

Recent research suggests that in addition to affecting the red-ox potential of cells, vitamin C also plays a role in regulating the epigenetic processes in the cell nucleus by affecting the activity of enzymes involved in active DNA demethylation. Our project will be the first to examine the molecular mechanism of how vitamin C affects human sperm. The study will be conducted in two experimental models: i/ *ex vivo*, where selected spermatozoa from samples with normal and abnormal sperm parameters will be incubated with vitamin C at different concentrations; ii/ *in vivo*, where patients with fertility disorders accompanied by local oxidative stress will be supplemented with vitamin C for three months. In both experimental models, we will assess a wide range of oxidative stress parameters, sperm chromatin integrity, and the extent of epigenetic modifications such as 5-methylcytosine and its oxidized derivatives in sperm DNA. We aim to identify differentially methylated loci in the sperm genome and examine the level of expression of genes/proteins involved in active DNA demethylation and active transport of vitamin C across sperm cell membranes. The research will use high-throughput multi-omics tools like mass spectrometry and microarrays. This modern research approach will allow us to find out whether sperm exposure to exogenous vitamin C limits alterations in sperm chromatin structure, reduces the number of broken DNA strands, normalizes the methylation/demethylation processes, and to what extent the observed differences in the methylome depend on oxidative stress in the semen as well as the ascorbate concentration inside spermatozoa. The results of this groundbreaking research may be useful in the development of new treatments for male infertility using use low-molecular-weight antioxidants.